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MARINE BIODETERIORATION.(U)
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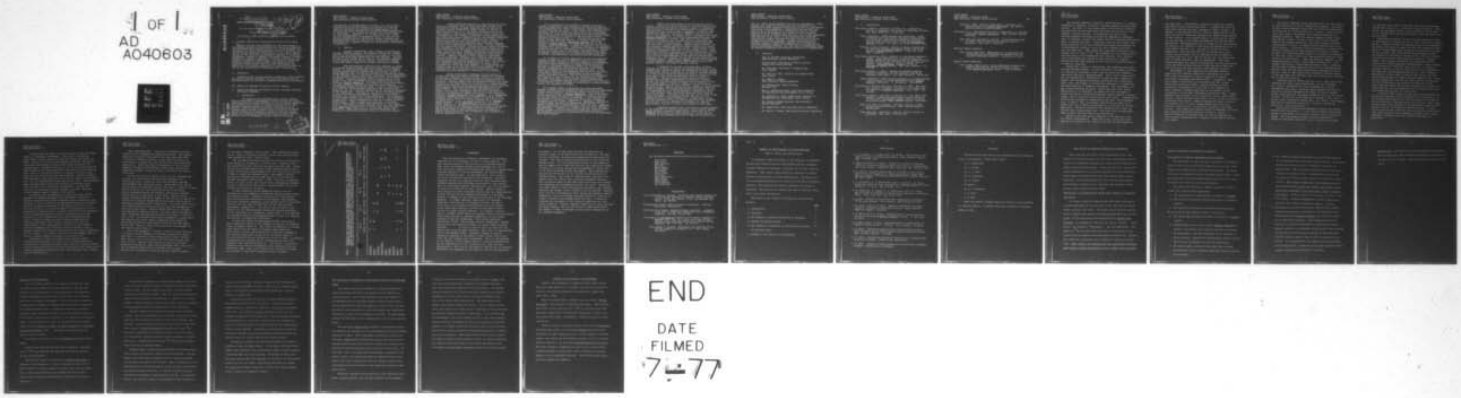

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Title: Marine Biodeterioration.

FINAL REPORT,
1 Oct 68-30 Sep 73.

For the Period October 1, 1968 - September 30, 1973

Contract: N00014-67-A-0251-0006

Contractor: Duke University, Durham, North Carolina

I. Summary Information Concerning the Overall ONR Program

Support has been made available for the continuation of a multifaceted program in biodeterioration, involving studies on the mechanisms which control molting, rate of development, metamorphosis, and sexual differentiation in larvae and adults of the Cirripedia (barnacles) and Brachyura (true crabs); investigations on the nutrition and digestive processes in Teredinids (shipworms); studies on temporal and spacial patterns in species abundance in subtidal, epibenthic (fouling) communities; and, research on barnacle ultrastructure, including an examination of short intervals of shell growth, the conditions which apparently effect growth, and the further development and application of cultured techniques for additional EM studies as well as an investigation of the metabolism of barnacles.

II. Objectives

Inasmuch as the overall program is composed of three separate but related research projects, the individual objectives and detailed results are included as separate portions of this report.

III. Reports on Separate Projects of Overall Program

A. Studies on Molting and Growth in Larval and Adult Barnacles and Larval Decapods

1. Objectives

To determine the mechanisms which control molting, rate of development, metamorphosis, osmotic balance and sexual differentiation in larvae and adults of the Cirripedia (barnacles) and Brachyura (true crabs), to continue studies on the adult barnacle endocrine system and further define the sites of activity, and to study the interaction of different endocrine systems within the same animals as well as well as the interaction of extracts of endocrine systems of different groups within the Crustacea and insects and their possible affect on molting, rate of development, growth, osmotic regulation,

reproduction, and metamorphosis of larvae from other groups. A second portion of this project was designed to study the nutrition and digestive processes of Bankia gouldi, of the family Teredinidae, wood-boring bivalve molluscs commonly known as "shipworms." The program was designed to provide answers to three general questions: (1) to what extent and in what way are shipworms able to metabolize wood?; (2) what are the ecological-physiological interactions between shipworms and microorganisms?; and (3) what are the relative contributions of wood-boring and suspension feeding to the metabolic requirements of shipworms?

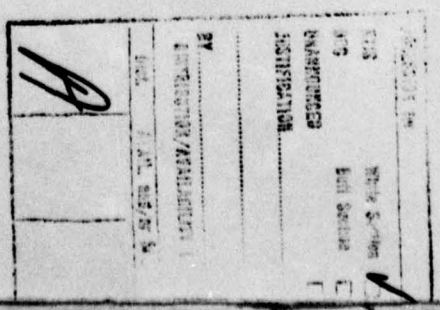
2. Results

Through collaboration with a number of postdoctoral fellows, graduate students, and research technicians, research has led to the publication of results in a number of the categories described under objectives (see Publications). Details of each of the studies may be found in the published documents. For the purpose of the report, however, significant findings within each general area of the project are given as follows.

Many physiological and behavioral responses in marine crustaceans change markedly during the course of one intermolt period. To take such variations into account, and to explore the mechanisms which may regulate the multiprocess itself, it became apparent that it would be necessary to identify the stages of the intermolt cycle in barnacles, preferably using morphological or physiological criteria which would not result in the death of the animal. From the research involving three members of our group, it has been determined that the intermolt cycle of the barnacle, Balanus amphitrite, is divided into three post-ecdysial, one inter-ecdysial, and four pro-ecdysial stages based on integumental changes within the cirri. Stage A is characterized by a seemingly single-layered exoskeleton and tortuous cirri. Stages B_1, B_2, and C are characterized by the increasing thickness of the endocuticle. Stage D_0, D_1, D_2, and D_3 are characterized by the progression of setogenesis, formation of the new exoskeleton, and resorption of the old endocuticle. The durations of the intermolt stages have a wide variability. The integumental changes, both within and between the rami of an animal, progress synchronously. These criteria will allow the use of live, intact animals taken at random from laboratory or field populations. The duration of the intermolt cycles of adult specimens of B. amphitrite varied from 1.5 to 23.5 days under constant conditions. There seems to be no correlation between the duration of successive intermolt cycles of individual animals.

Having established methods for identifying the stages within one intermolt cycle of adult barnacles, studies were initiated to develop in vitro techniques for the culture of Cirriped tissues, primarily to permit studies on the way in which certain cells, i.e., the cement secreting cells of the adult barnacle, develop and synthesize the cement itself and, to determine if this process of synthesis and proliferation of the cement ducts may be regulated by the same hormonal mechanisms which control molting. Various components were tested as potential nutrients in media for barnacle tissue cultures. Bovine embryo extract and yeastolate were favorable additions for both cell outgrowth and organ cultures. Glucose, however, did not improve the medium. In organ cultures, mantle parenchyma were obtained for 25 days, ovarioles and cement glands for at least seven days with no change in structure and organization. Mature oocytes and young immature oocytes were maintained for a minimum of seven days, and oogonea retained a high mitotic activity. Immature oocytes in later stages of development were not maintained. Extensive cell outgrowth forming large surface spreads and with cells showing high mitotic activity were obtained from attached explants for 18 days. The initial cell migration consisted of epithelial-like cells, later, fibreglas-like cells were most abundant. A predominantly non-ovarian origin of the migrating cells seems probable. It is suggested that the ovarian part of cell donation may be increased by early dispersion of the cells in the explants.

Having established a technique for the culture of tissues extirpated from adult barnacles, studies were designed to describe a sequence of events in cement secretion occurring at the cellular and tissue level during one intermolt cycle of barnacles. Previous reports had not determined whether the cement secretion of the gland cells is a continuous or a discontinuous process which might be correlated with the physiological condition of the animal or activated by hormonal mechanisms within the adult animals. From this work it has been determined that regions of secretion accumulation and secretion synthesis do exist in the cytoplasm of cement gland cells of two species of barnacles, Balanus amphitrite and B. eburneus. In B. amphitrite, secretion accumulation was at its maximum in Stage B_1 of the intermolt cycle, decreasing during Stage B_2, was zero in C and increased from B_1 to D_2. A similar variation in B. eburneus seemed probable. In B. amphitrite maintained in an aquaria before fixation, no secretion accumulation was observed in cement gland cells, irrespective of the molt stage of the animal. The study suggests that detectable secretion accumulation in cement gland cells of adult barnacles is not necessarily a species characteristic but may be dependent upon endogenous as well as exogenous factors.



As an outgrowth of the studies previously mentioned, a number of experiments were designed to determine the capacity for biosynthesis of a number of compounds, predominantly sterols, in both barnacles and crabs. Other studies were conducted to determine the way in which free amino acids may change during the course of a molt cycle and the extent to which these changes may be regulated by the removal of one particular site of endocrine activity in crabs, i.e., the eyestalk and the sinus gland, exorgan contained therein. A dietary requirement for sterols in the blue crab, Callinectes sapidus, and in the barnacle, Balanus nubilus, was established through the use of isotopic tracer techniques. A number of aspects of the research indicated that both of these crustaceans are unable to synthesize squailine and sterols from acetate or from mevalonate. Changes in the free amino acids in developing crustacean larvae were described, including the way in which these changes may relate to molting, osmoregulation, and possible hormonal mechanisms.

In an effort to further understand the interrelationships between a number of sites of endocrine activity in Crustacea, experiments were designed to describe changes in the androgenic gland (the gland responsible for sexual differentiation and maturity in Crustacea) related to removal of the eyestalks and the x-organ sinus gland complex associated with molting control and inhibition in larvae and juvenile crabs. It was shown that removal of both eyestalks from larvae of two species of Brachyura resulted in an hypertrophy of the androgenic glands of juvenile animals. This is accompanied by hyperplasia and increase in size of cells; at the ultrastructural level, the gland cells show a well developed granular endoplasmic reticulum, mitochondria grouped in a juxtanuclear area, rounding of the nucleus, and an increase of accumulation of electron dense material in intracellular spaces.

In a further effort to determine the interrelationship between a number of physiological processes and hormonal mechanisms, studies were designed to investigate osmotic balance and its control in the barnacle, Balanus improvisus. From these studies it was shown that this particular species of barnacle survives well in freshwater as well as in seawater, in part because of its ability to regulate the hemolymph and in part because of regulation of cell volume. Adults of B. improvisus osmoconform in waters above 500 mOsm and osmoregulate in more dilute seawaters, showing strict homoiosmoticity below 100 mOsm. Hemolymph and maxillary gland fluid are isosmotic and have equal chloride concentrations. Seventeen free amino acids were found in thorax muscle tissues in amounts varying with hemolymph osmolality. The intermolt cycle does not significantly influence the parameters which were measured.

Within the final objective of this portion of the project, studies to determine the extent to which hormones from insects and Crustacea may be related, a number of experiments were designed to determine the effectiveness of ecdysone and synthetic analogs on the larvae of Limulus, a Xiphosura which demonstrates many characteristics common to the insects and also to the Crustacea. Molting in Stage I horseshoe crab larvae, Limulus polyphemus, can be induced by polyhydroxysteroids that stimulate molting in insects. The effect of the injected steroid in shortening the intermolt period is dependent upon dose and time of a natural molt. Further studies indicated that two insect molting hormones, Alpha and Beta ecdysone, and two synthetic ecdysone analogs are highly effective in stimulating molting in horseshoe crab larvae. Effectiveness of the ecdysone is dependent upon time in the intermolt cycle. Large doses were found to be necessary to induce molting early in the cycle and no larvae completed ecdysis, but late in the cycle only 0.02-0.06 micrograms/gram of the natural ecdysones were necessary for a 50% response and over 80% of the injected larvae completed ecdysis. Responses of Limulus larvae to the ecdysones in some synthetic analogs suggest basic similarities to the chemical control of molting in certain insects.

In addition to the work described above, studies continue in an effort to provide an accurate description of the changes which take place during the development of organ systems in developing larvae, primarily in an effort to develop concepts as to how certain endocrine mechanisms develop and may be interfered with at some point during early larval or juvenile development. One portion of this work involved efforts to describe two prominent morphological features of Cirriped larvae, the "sac and frontal filaments" and determine if there may be physiological functions, especially the possibility that the filaments may serve as receptor organs in the developing barnacle larvae. Earlier studies on this particular aspect of larval Cirripedes has created considerable confusion, not only in terminology but also the phylogenetic relationships which may exist between these particular organs and those found in other crustaceans. From this study the "sac" at the base of the frontal filament in the nauplier stages can definitely be identified as the "Organ of Bellonci." A description of the cellular components of the sac and filaments has been provided but, as yet, it is not possible to attribute any specific physiological function to either the filaments or the organ.

A separate section within this project included studies on the digestion of cellulose and wood in the shipworm, Bankia gouldi Bartsch, conducted largely by Mr. Robert Dean as a candidate for the Ph.D. at Duke University. A model for the structure of the secondary cell wall of wood was presented, as

well as a model for mechanisms involving its degradation. Invertebrate wood ingesters increase cell wall accessibility by grinding the wood; enzymatic delignification has not been studied other than in microorganisms. Experiments were designed and carried out to determine if the marine wood boring, bivalve molluscs known as shipworms were capable of wood digestion, especially of the cellulose fraction and if so, by what mechanisms. From the work of Mr. Dean, it was concluded that shipworms significantly digest cellulose and wood, including the native crystalline cellulose fraction. Hemicellulose digestion is probably similar to mechanisms described in microorganisms. Cellulose digestion could not be demonstrated in vitro and may involve mechanisms different from that seen in cellulolytic fungi. An alternate mechanism using oxidation reactions is hypothesized.

3. Personnel

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III. B.
ONR Final Report
John Sutherland

The fouling community at Beaufort, North Carolina, is a complex assemblage of hydroids, tunicates, bryozoans, sponges, and associated species. The object of our studies since 1971 has been to identify and understand the processes which determine the structure of this communty in space and time. To accomplish this goal I have followed community development beneath the Duke Marine Laboratory dock, on unglazed, ceramic tile plates (232 cm^2) suspended horizontally about .3 m below low water. Percentage cover for each species that settled and grew on the lower surface was estimated periodically by a point-sampling technique, using 75 points randomly positioned over the plate area. This number gave repeatable estimates of percentage cover to within $\pm$ 5%. The technique was also nondestructive, allowing plates to be resubmerged after sampling. Only the basal area of attachment was counted as being occupied by a species, and greater than 100% coverage for all species was possible because of epizooic overgrowth.

During 1971, series of three plates were submerged each month from May thorugh November. In 1972 (and 1973), the number of plates in a series was increased to four, and new series were submerged each month from April through November. Only a few of these series will be reviewed here. Additionally in 1972 and 1972, competitor-removal and fish-predator-exclusion experiments were conducted in April, July, and October to evaluate the importance of competition and fish predation on community development. The experimental unit was a rack containing 20 plates. Within each rack, four plates were controls (= no removals), and there were four treatments (= removal by hand of a potentially dominant sessile species or species group), each of which was carried out on four plates. Treatments and controls were assigned at random within the rack. Each experiment (e.g., in April, July, or October) consisted of two racks of 20 plates each, one of which was enclosed in a nylon fish net ($\frac{1}{4}$-inch mesh size). Identical treatments were conducted both inside and outside these fish-exclosure nets. ONly data from the experiments begun in April 1972 and 1973 are considered here.

Plates in the same series, submerged at the same time, were not sampled simultaneously. Thus, data from a given plate often applied to a different time interval from that of other plates in

the same series. Data within a series were pooled by a method described elsewhere (Sutherland 1970). I assumed the events on each plate occurred uniformly throughout a sample interval. For example, if the percent cover of a species changed by 30% in 30 days, the rate of change was assumed to be 1%/day during each day of that interval. In a computer memory, an array was set aside for each species on each plate and numbered for each day after submergence. The appropriate rate of change ($\pm$) for each species was placed in the elements of the array representing a day in a given sample interval, e.g., from day 70 to day 100 after submergence. When this was done for all species on a given plates, the percentage cover of each species was estimated at arbitrary intervals (e.g., day 30, 60, 90, etc.) by summing the rate of change from day 1 (day of submergence). Data were transformed using the angular transformation, and for each series a mean and 95% confidence interval (Sokal and Rohlf 1969) were then calculated for each species at each arbitrary interval.

Development in this community was extremely variable. Associated with seasonal temperature fluctuations (of about 25°C) there were distinctly seasonal patterns of species recruitment (Sutherland and Karlson 1973). In addition, there were dramatic differences in recruitment from year to year (Sutherland and Karlson 1973). Seasonal and year-to-year variations in abundance were also seen in the community of adult organisms, although these were not as dramatic as the variability in recruitment (Sutherland and Karlson 1973). Common hydroids were *Tubularia crocea*, *Eudendrium carneum*, and *Pennaria tiarella*; common tunicates were *Styela plicata*, *Ascidia interrupta*, and *Molgula manhattensis*; common bryozoans were *Bugula neritina* and *Schizoporella unicornis*; common sponges were *Microciona prolifera*, *Haliclona* sp., *Halichondria bowerbanki*, and *Mycale cecilia*. Also common were several species of barnacles (*Balanus*) and a serpulid polychaete (*Hydroides dianthus*). Below, I restrict attention to species which, at some time during the time interval considered here, occupied at least 10% of the area of a series.

The series submerged in May and August 1971 provide examples of the variability in initial community development. The series of three plates submerged in May 1971 was initially dominated by *Tubularia* and to a lesser extent by *B. neritina*. *Bugula neritina* has a small zone of attachment and is underestimated by our sampling technique. These two species produced a dense canopy some 180 mm in depth. In June, *Styela* (and *Ascidia*) settled beneath this canopy; during July, the series became essentially a monoculture of *Styela*, a condition which persisted until the end of October. I veiw this *Styela* monoculture as a stable point becuase it persisted for some period of time (about 4 months) in spite of forces potentially capable of altering its structure. In this case, these "forces" were represented by potential larval recruits of other species. Thus, new substrate submerged in June became dominated by *Ascidia* (which kept settling after *Styela* stopped) and *Pennaria* (Sutherland and Karlson 1973). Similarly, the July series becmae dominated by *Balanus* sp. (Sutherland and Karlson 1973), and the August series became dominated by *Schizoporella*. None of these species was recruited to the May series at the same time; this indicates that their larvea were "filtered out in some way by *Styela*.

Toward the end of October and in November, many *Styela* died, rendering the tightly grown-together mat of *Styela* unstable. As a result, essentially all *Styela* sloughed off, leaving considerable free space, which was again dominated by *Tubularia*. *Tubularia* persisted throughout the winter (a time of minimal larval recruitment) but was again invaded by *Styela* in the spring of 1972. In contrast to the behavior in spring of 1971, *Styela* did not form a monoculture during 1972. As a result, other species were able to invade the series (e.g., *Pennaria*, *Eudendrium*, *Balanus* sp., and *Schizoporella*).

The series of three plates submerged in August 1971 became dominated by *Schizoporella*. I view this as another stable point because it persisted (for at least a year) in spite of potential change. The most dramatic evidence for this resistance to change was the genral absence of *Styela* in this series during

the spring of 1972, when at the same time *Styela* was invading the May 1971 series (i.e., *Tubularia*) very successfully. Indeed, except for the August series, *Styela* invaded all of the 1971 series (May-November) during the spring of 1972 (Sutherland and Karlson 1973).

Experiments conducted in April 1972 shed additional light on the production of these two (*Styela* and *Schizoporella*) stable points. Controls outside the fish-exclosure net are comparable to the May 1971 series, except that they were submerged in April. However, the May 1972 series developed in essentially the same way as the April 1972 series (Sutherland and Karlson 1973). In contrast to the events of spring of 1971, *Tubularia* did not settle heavily during 1972 and did not produce an extensive canopy. The controls outside the net became dominated by *Schizoporella*, which persisted through December, again in spite of the presence of potential larval recruits. In dramatic contrast, controls inside the fish-exclosure net, submerged at the same time, became dominated by *Styela*. *Styela* formed a monoculture, as was formed on the May 1971 series, and this monoculture persisted for about the same length of time. After *Styela* sloughed off, the area was taken over by *Schizoporella* in 1972, as opposed to the regrowth of *Tubularia* in 1971. *Styela* removals inside the net were dominated by *Schizoporella*, indicating that the nets themselves had no adverse effects on recruitment and survival of *Schizoporella*.

I intepret these resutls to mean that fish predation is a potentially important source of mortality to young *Styela*. If the foraging efficiency of the fish is reduced at the time of *Styela* recruitment, then many more *Styela* survive. Thus, *Styela* survived well in the absence of nets during the spring of 1971 because *Tubularia* had formed an extensive canopy. In 1972, *Styela* survived only inside the fish exclosure nets and on the 1971 series (except the one submerged in August) where other organisms, including *Tubularia*, provided some degree of protection.

I should point out that I have been unable to duplciate these results in subsequent experiments. There was no significant recruitment of *Styela* during the July and October experiments in 1972. *Schizoporella* was the most abundant colonizer, and initial recruitment and growth were equal both inside and outside the exclosure nets. Probably *Schizoporella* is not a preferred food for fish because of its encrusting growth form and heavy calcification. Thus, there is no reason to expect an increase in survival when *Schizoporella* is protected from fish predation. During the April 1973 experiment, *Styela* did not settle heavily inside the exclosure net. The net inadvertently became fouled with *Styela*, which probably prevented additional larvae from reaching the enclosed plates. Thus, no test of the fish effect was possible. In July 1973, *Schizoporella* was again the most abundant colonizer, and there is no necessary reason to expect a fish effect.

I conclude that the production of these two stable points depends on a complex and potentially hierarchical pattern of switches involving both larval recruitment and, occasionally, fish predation. Thus, a *Schizoporella*-dominated system can be produced directly by larval recruitment in the absence of *Styela*, as in the fall of 1971, or it can be produced in the presence of *Styela* recruitment if fish are able to forage effectively on the substrate. A *Styela*-dominated system can result if previosuly recruited species (or a net) afford protection from fish predation and *Styela* is able to invade the area occupied by these species. *Tubularia* satisifies these criteria; *Schizoporella* does not. Additionally, *Styela* larvae must be available in the plankton. Initial development into one stable point or another is thus a highly stochastic process with the order of events just as important as their nature. As a result, the history of development must be known to explain community structura at any given time.

Maintenance of these two stable points appears largely determined by the ability of either *Styela* (in monoculture) of *Schizoporella* to exclude potential larval recruits. Direct evidence of this process for *Schizoporella* has been found in the *Schizoporella* removal experiments conducted in April 1972 and April 1973, outside the fish exclosure net.

April 1972 experiment: *Schizoporella* dominated all four control plates throughout the observation period. This species generally occupied at least 50% of the area until June 1974. In contrast, except for *Balanus* in June 1972 (18% cover), no other species occupied more than 10-13% of the area during the same period. After June 1974, the abundance of *Schizoporella* dropped to 25-28% and by December several other species were moderately abundant, especially *Styela* (15% cover) and *Balanus* (15% cover).

On the *Schizoporella* removal plates, *Balanus* abundance was variable, but was greater than 18% cover throughout the observation period, except for June 1973 (8% cover) and June and September 1974 (5% and 9% cover). By December 1972, *Mycale* was the other most important species (39% cover), joined to a lesser extent by *Microciona* (11% cover) and by *Ostrea* (9% cover). *Mycale* disappeared during the spring of 1973 and was replaced briefly by *Styela* (33% cover in June 1973). However *Styela* almost disappeared during the summer of 1973 and *Balanus* and *Ostrea* collectively occupied 54% and 60% of the area on this series in September and December 1973, respectively. *Microciona* and *Haliclona* were common epizooic species on *Balanus* and *Ostrea*through most of the observation period. Finally, during 1974 *Styela* reinvaded and remained the dominant species. For some reason this species didn't disappear from these plates during the summer of 1974 as it did in 1973. By December 1974, species abundances on both control and removal plates were similar; treatments were stopped by March 1974 and the two series were "tested" by the same larvae during 1974. However, *Schizoporella* was still less abundant on the removal plates (Table 1).

April 1973 experiment: In 1973, *Schizoporella* was initially a relatively minor component of the control group (only 10% by June 1973), in contrast to the control group in 1972. By June 1973, *Bugula*, *Styela*, and *Ascidia*, were all more abundant than *Schizoporella*. However, both species of tunicates declined dramatically during the summer and by September 1973, *Schizoporella* was the dominant (28% cover) on the 1973 control group. *Schizoporella* continued to be one of the most abundant species

on this group throughout 1974 (% cover > 19%) although *Haliclona, Bugula, Styela, Botryllus, Hydroides, Balanus* and *Corophium* were also abundant (% cover > 10%) at times.

The treatment group was initially very similar to the control group except for the absence of *Schizoporella*. However, there was significantly more *Tubularia* and *Balanus* by June 1973. *Styela* and *Ascidia* declined dramatically in the treatment group as well as the control group during summer 1973, but *Schizoporella* was not allowed to invade the treatment group. By December *Tubularia* was the most abundant species (20% cover) but *Styela* was the dominant throughout 1974 (% cover > 17%). *Haliclona, Eudendrium, Bugula, Ascidia, Balanus* and *Corophium* were periodically abundant (% cover > 10%) during 1974 as well, and by December *Styela* was covered with a layer of *Botryllus*.

In each experiment one-way ANOVA was used to test for differences in the abundance of individual species between control and treatment groups. Percentage cover data for each species from each plate were mornalized with the angular transformation. Tests were conducted biannually on all species except *Schizoporella*, starting with June 1972 and June 1973 for the 1972 and 1973 experiments, respectively. *Schizoporella* was included in the analysis for the 1972 experiment during 1974 because treatments in this experiment were terminated in March 1974. I have reported the arithmetic means of nontransformed data in Table 1 to indicate the direction of significant differences.

AT some time during the observation period, *Schizoporella* had a significant effect on the abundance of many of the important community members (Table 1). This effect was generally to exclude other speices from the space it occupied; when significant differences occurred a given species was always more abundant on the treatment group where *Schizoporella* was absent (Table 1). The only exception was for *Schizoporella* itself in 1974. It remained significantly less abundant on the treatment group even after treatments were terminated (Table 1). *Schizoporella* larvae were abundant during 1974 and did dominate newly submerged substrate during that time. Thus, while *Schizoporella* was able to exclude many species, it was itself excluded by other residents.

Table 1. Arithmetic means of percent cover which differed significantly ($p < .05$) between experimental groups. Differences were evaluated with one-way ANOVA after individual plate data were transformed with the angular transformation (Sokal and Rohlf, 1969). Data from the 1973 experiment marked with an "*". Rest of data from the 1972 experiment. Note that transformed means are not reported.

	Dec 72		Jun 73		Dec 73		Jun 74		Dec 74	
	Control	Sch Rem	Control	Sch Rem	Control	Sch Rem	Control	Sch Rem	Control	Sch Rem
Microciona	0	10								
Mycale	0	38	0	16						
Tubularia			11*	25*	6*	20*				
Schizoporella							27	9	26	6
Styela							8	36	10*	36*
Ascidia					0*	6*	1*	9*		
Hydroides					2	8				
Ostrea	0	8	0	10	2	22			0	9
Balanus	1	17	1 5*	8 24*	12	38				

DISCUSSION

The starting point of community development on unoccupied substrate is the recruitment of larvae to that substrate. The most striking feature of this process is its unpredictability. Thus larval recruitment patterns varied markedly from year to year and initial community development was essentially unpredictable; different patterns of initial development were observed both within and between years. As discussed above, these patterns were also occasionally affected by fish predation. Instead of preparing the way for subsequent arrivals, most resident adults strongly inhibited the recruitment and growth of other species. This is an obviously adaptive characteristic, for to be overgrown is often to be killed or at least starved. Species varied in their ability to resist subsequent invasion and the ranking process is made difficult by the inherent differences in life spans. However, an example of this difference can be seen in the following gradient: 1) "almost immortal" *Hydractinia*, 2) "strongly resistant" *Schizoporella*, 3) strongly resistant, but short-lived *Styela*, 4) essentially nonresistant *Ostrea* and *Balanus*. On the other hand, species of larvae also differed in their ability to invade occupied substrate. For example, *Botryllus*, and to a somewhat lesser degree the sponges *Haliclona* and *Halichondria* almost never settled on unoccupied substrate, but were common invaders of "mature" assemblages. The solitary tunicates, particularly *Styela*, often invaded "mature" assemblages. However *Schizoporella* was essentially unable to invade already occupied substrate in spite of the fact that the larvae of this species were often extremely abundant. Thus, after an unpredictable initial developmental phase, subsequent changes in species composition depended on the degree to which larvae could "force" their way into existing adult assemblages. This in turn depended on the identity of the resident adult(s), which was unpredictable, and the identity(ies) of the invading larvae, which was also unpredictable. As a result, the direction and the rate of community development was dependent on the order of invasion and impossible

to predict. For the most part diversity did increase, at different rates, to an equilibrium level of about 2.5, but this was not inevitable; one plate was monopolized by *Hydractinia* for 2½ years. The species composition could also be very different between plates. The only predictable feature of the changes in species composition is that they did not stop; significant changes occurred on all plates (except the one dominated by *Hydractinia*) in the years following initial development. These changes were generated by the arrival of new recruits and the mortality of resident adults. Both kinds of "perturbations" occurred on an annual basis, the latter often producing unoccupied substrate. This unoccupied substrate was most often occupied by new recruits, thus beginning the developmental process over again. Because of the annual nature of these perturbations the plates were probably observed long enough to see a climax if one were present. However, given the type of perturbation which caused the changes in species composition, larval recruitment and adult mortality, there is no reason to believe these changes would ever stop. I therefore conclude that succession in the classical sense does not occur in this community. Instead, community composition in space and time is always changing, the rate and direction being determined by the regime of larval recruitment and the species composition of the resident assemblage.

PERSONNEL

The following persons participated with me on this project:

Andre Aucin
Mary Berry
Mike Corcoran
Ann Dean
Betsy Dupree
Marty Farmer
Tom Fisher
Peter Friday
Tim Green
Wayne Harrington
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Penny Hooper
Walter Nelson
Pat Sutherland

PUBLICATIONS

1973 (with Ronald H. Karlson) Succession and seasonal progression in the fouling community at Beaufort, North Carolina. 3rd Int. Cong. Mar. Corros. & Foul. Northwestern Univ. Press. pp. 906-929.

1974 Multiple stable points in natural communities. American Naturalist, 108:859-873.

1976 (with B.A. Menge) Species diversity gradients: synthesis of the role of predation, competition and environmental stability. Am. Nat. 110:351-369.

1977 Effect of Schizoporella removal on the fouling community at Beaufort, N.C. In: B.C. Coull (ed.) Ecol. of Mar. Benthos, Belle W. Baruch Institute for Marine Science Symposium, May 7-10, 1975. pp. 155-176.

(with Ronald H. Karlson) Development and stability of the fouling community at Beaufort, N.C. Ecol. Monogr. (in press).

Studies of calcification of marine organisms

Karl M. Wilbur and collaborators

An important aspect of fouling is the formation of skeletons of calcium carbonate by marine invertebrates and the attachment of these skeletons to surfaces. Barnacles fall in this class of organisms. Other marine invertebrates are important in fouling because of penetration of marine installations. The penetration is accomplished by movements of a portion of the calcium carbonate skeleton. The marine boring molluscs represent this group of organisms. The focus of our research has been on skeletal formation in barnacles and molluscs.

The report of our studies is divided into the following sections:

Publications

V. R. Meenakshi, P. E. Hare and K. M. Wilbur. Amino acids of the organic matrix of neogastropod shells. Comp. Biochem. Physiol. 39B, 1037-1044, 1971

B. Heatfield and K. M. Wilbur. Effects of a carbonic anhydrase inhibitor on skeleton growth and micro-structure in regenerating sea urchin spines. (Abstract) Amer. Zool. 12, (4), 516:718, 1972.

K. M. Wilbur. Mineral regeneration in echinoderms and molluscs. Ciba Foundation Symposium 11, Hard Tissue Growth, Repair and Remineralization, ASP (Elsevier Excerpta-North Holland), Amsterdam, pp. 7-33, 1973.

V. R. Meenakshi, P. L. Blackwelder and K. M. Wilbur. An ultra-structural study of shell regeneration in Mytilus edulis (Mollusca: Bivalvia). J. Zool., 171, 475-484, 1973.

V. R. Meenakshi, G. Donnay, P. L. Blackwelder and K. M. Wilbur. The influence of substrata on calcification patterns in molluscan shell. Calc. Tiss. Res., 15, 31-44, 1974.

F. Losada. Studies on growth and shell deposition in barnacles of the genus Balanus. Ph.D. Thesis. Duke University, 1975.

J. H. Waite, and K. M. Wilbur. Dalbergia polyphenols and shell formation in molluscs: preliminary results. Naval Research Laboratory Report. In press.

J. H. Waite and K. M. Wilbur. Phenoloxidase in the periostracum of the marine bivalve Modiolus demissus Dillwyn. J. Exp. Zool. In press.

J. H. Waite and C. H. Wang. Spectrophotometric determination of dodecyl sulfate with basic fuchsin. Anal. Biochem. In press.

J. H. Waite. Rosewood polyphenols alter phenoloxidase activity from the mantle of the marine bivalve Modiolus demissus Dillwyn. Pest. Biochem. Physiol. In press.

J. H. Waite. Calculating extinction coefficients of enzymatically produced o-quinones. Submitted for publication.

J. H. Waite. Effect of protein denaturants on molluscan o-diphenol-oxidase. Submitted for publication.

Personnel

We have had the good fortune of collaboration by an excellent group of researchers. Their names follow:

P. L. Blackwelder

Dr. G. Donnay

Dr. P. E. Hare

Dr. B. Heatfield

Dr. F. Losada

M. Markman

Dr. V. R. Meenakshi

J. H. Waite

C. H. Wang

Under the project, Freddy Losada has carried out and completed a doctoral thesis. J. Herbert Waite will complete his doctoral thesis in 1976.

The effects of rosewood extractives on shipworms

This study has its origin in the observation of Dr. Ruth Turner of Harvard University that the larvae of shipworms do not settle on or penetrate rosewood or on other wood treated with compounds extracted from rosewood. Later studies by Dr. Turner and others demonstrated that rosewood compounds are also effective in protecting or partially protecting woods against other fouling organisms. Our investigations have concerned the physiological and biochemical effects of two compounds from rosewood, obtusaquinone (OBQ) and obtusastyrene (OBS).

Inhibition of calcification of marine borer larvae by a rosewood extractive.

Dr. Turner's studies indicated that the larvae inhibited by rosewood compounds did not form their usual calcified structures (teeth, pallets, and cones). The teeth are necessary for boring. We have measured the effects of obtusaquinone-treated wood on the uptake of ^{45}Ca by larvae of the molluscan borer Lyrodus pedicellatus. As a measure of the action of OBQ on protein metabolism, the uptake of ^{3}H-glutamine was also determined. The uptake of ^{45}Ca was found to be more than 3 times greater in untreated wood as compared with obtusaquinone-treated wood. However, the metabolism of glutamine was unaffected in larvae on treated wood. These results have demonstrated that obtusaquinone-treated wood inhibits calcium metabolism of borer larvae without altering

protein metabolism as measured by glutamate.

The effects of rosewood compounds on phenoloxidase.

The substratum on which calcium is deposited in forming the shell of borer larvae and molluscs generally is an outer shell covering of tanned protein called the periostracum. The cross-linking of this protein involves the enzyme phenoloxidase. Rosewood compounds could conceivably inhibit calcification by interfering with the formation of the periostracum.

The study of the effects of rosewood compounds on phenoloxidase has involved three approaches:

1. The demonstration of phenoloxidase in larvae of Lyrodus.
2. The effects of obtusaquinone and obtusastyrene on phenoloxidase.
3. The properties and kinetics of molluscan phenoloxidase.

We now summarize the results on each of these phases.

1. The enzyme phenoloxidase was found to be present in Lyrodus larvae.
2. Phenoloxidase in the bivalve mollusc Modiolus demissus is stored in the mantle in an inactive form and is activated by chymotrypsin. The same activation can be expected of other molluscs. Normally the phenoloxidase would be activated following its secretion but not by chymotrypsin.
3. The active enzyme is found in the outer shell covering (periostracum) where it probably crosslinks proteins secreted by the mantle.

4. The rosewood compound obtusaquinone is altered by extracts of the molluscan mantle and outer shell covering. The altered obtusaquinone then actis as a competetive inhibitor of phenoloxidase. This inhibition probably prevents the cross-linking of the periostracum. Obtusaquinone also enhances the toxicity ov obtusastyrene toward the periostracal phenol-oxidase. These results demonstrate that obtusastyrene and obtusaquinone from rosewood may inhibit borer larvae by preventing the formation of the substratum on which calcification occurs. Further details will be found in Waite, H. and K. M. Wilbur, Dalbergia polyphenols and shell formation in molluscs, Naval Research Laboratory Report. In press.

5. The properties of the phenoloxidase affected the rosewood extractives have been investigated in the periostracum of the marine bivalve Modiolus demissus from which the enzyme can be obtained in quantity. This exoskeletal structure contains two forms of phenoloxidase solubilized by sodium dodecyl sulfate: a low molecular weight protein (about 80,000) of high specific activity and a protein of extremely high molecular weight (greater than 2×10^7) of low specific activity. It is proposed that the enzyme activity associated with the protein(s) of high molecular weight represent phenol-oxidase covalently immobilized by o-quinones.

Publications. Four papers have been accepted for publication and two additional ones have been submitted for publication on this phase of our studies. These are listed elsewhere in this report.

Studies of barnacle growth

One of the major aspects of our program has been the study of barnacle growth under controlled conditions. This was made possible by the development of culture apparatus for maintaining constant temperature, constant flow of sea water, and utilizing sea water as a natural food supply. We have been able to provide a comprehensive picture of barnacle growth from larval settlement to an advanced adult stage. In addition, experimental studies on growth under controlled conditions have been made and compared with growth in the natural environment. The results are summarized below. Further details are given in a doctoral thesis by Freddy Losada entitled Studies on growth and shell deposition in barnacles of the genus Balanus, 1974 available on microfilm from the Duke University Library.

The growth of the shell in several Balanus species was investigated.

Growth rates were measured from shell dimensions, incorporation of ^{45}Ca into the shell and from the formation of markings on the calcified plates.

The absolute growth in basis area in Balanus improvisus was dependent upon temperature. A rise in temperature from 15°C to 20°C resulted in a larger increase in growth rate than the respective increase when temperature was raised from 20°C to 25°C. Growth rates of young barnacles were 1.5 times faster during the nighttime.

The allometric analysis of several shell dimensions in relation to the rostro-carinal length of the basis showed some changes of shell shape. As the barnacle grew older, it became taller in relation to the basis length. Also, the basis became more circular and the operculum orifice smaller in relation to the basis length. Similar allometric trends were found in the size analysis of the population and the growth history of the individual.

Calcium content of the mantle was greater than other body regions. This is in agreement with the recent finding that intracellular $CaCO_3$ crystals are formed in this tissue (Watabe, unpublished). Calcium deposition occurring in few hours on the opercular plates of Balanus amphitrite was measurable using ^{45}Ca. The rate of deposition was inversely related to size (or weight of the opercular valves) and showed changes throughout the intermolt cycle. Barnacles deposited more ^{45}Ca during early postmolt stages and less at premolt stages.

Barnacle shell is deposited periodically in increments which form a pattern of concentric bands on all shell plates. The suggestion that those bands represent cycles of growth associated to successive intermolt cycles (Darwin, 1854) is supported in this investigation by a detailed analysis of the process of band formation and the factors affecting it. The rate of band formation increased with temperature and decreased with age. In young barnacles, this rate was found to be equivalent to the frequency of

exuviation of the body. Different stages in the formation of a basal band corresponded closely to stages of the intermolt cycle. Also, in the present investigation basal bands were used as a measure of growth rate.

Microscopic growth lines within a single band are formed on the external surface of the wall plates. These lines are of a subdaily nature. They do not represent a periodicity associated with tides. The distance between successive growth lines was related to temperature and growth rate. Several patterns of growth line deposition were described. Growth lines are probably the result of the interaction of several biological and environmental factors acting together. Except for the possibility to describe qualitative changes in growth rate, they do not have the same predictive value as growth lines on the molluscan shell.

Variations in texture were observed throughout the internal surfaces of wall and basal plates. At the basis, the inner lamina showed under polarized light two distinct regions on which crystalline differences were distinguished. Differences between outer and inner laminae were also described in the basis. On the internal surfaces of the wall plates, the growing basal edge was formed by crystalline columnar structures, whereas other regions showed round, irregular or polygonal crystals.

The influence of substrata on calcification patterns in molluscan shell.

The general form of the skeletons of calcium carbonate of fouling organisms may follow the contours of the material on which they grow. On the submicroscopic level, the crystals of the skeleton have an arrangement which is often characteristic of the species which forms the skeleton. At this level, one can ask whether the substratum on which the crystals are formed also influences the form of the crystalline material. No experimental studies had been made on this problem prior to the study reported below.

The land snail Otala lactea provides an experimental system for studying the influence of the substratum on the crystal characteristics of shell. After experimental removal of a portion of the shell, Otala deposits crystalline $CaCO_3$ in the form of fine grained aragonite and large grains of calcite on an organic substratum secreted by the mantle which lies next to the shell. We have found that if an experimental substratum is inserted in the area of repair, the crystalline material deposited will be different from normal regeneration and will conform closely to the microtopography of the surface of the substratum inserted in the repair area.

Substrata, including the periostraca of four species of molluscs, surface replicas, and the outer membrane of the eggshell

of the hen, have been inserved in the shell repair in Otala, and the topographical patterns of deposition of calcium carbonate have been observed by scanning electron microscopy. The mineral patterns formed on all inserted substrata conformed to the micro-topography of the surfaces and were distinctly different from that of the normal shell regeneration. The topography of the normal outer mineral surface of the shell of four species of molluscs were observed to conform to the topography of the periostraca on which the crystalline shell is deposited. It is inferred that the topography of the outer mineral surface of the shell probably results from the interaction of crystals of aragonite and calcite growing in an organic matrix and in contact with the periostracum. Normal shell of Otala consists of fine-grained aragonite in highly preferred orientation. Regenerated shell contains calcite grains in random orientation and aragonite, usually in random orientation. The aragonite-to-calcite ratio varies widely and appears to be independent of experimental substrata placed in the area of repair.

Studies on calcification in echinoderms

Studies were undertaken on regenerating sea urchin spines which have been shown to be a valuable tool for the quantitative study of calcification and growth in echinoderms (Healtfield, Biol. Bull., 1970).

Rates of regeneration of spines of the sea urchin, Arbacia punctulata, were studied in long-term experiments. These studies show (Fig. 5) that growth at 15° - 16°C is initiated about four days after experimental fracture and continues at a linear rate up to about forty days. Thereafter, a gradual decline in growth rate occurs.

Studies by Wilbur and Jodrey (1955) on the oyster Crassostrea and by Costlow (1959) on the barnacle Balanus demonstrated the inhibition of shell formation by the carbonic anhydrase inhibitor Diamox. Our studies on the effects of Diamox, 10^{-5} M, on regeneration of spines of the sea urchin Strongylocentrotus purpuratus have demonstrated a retardation in initiation of calcification, a marked decrease in growth rate, and an alteration in the morphology of the regenerating skeleton. These effects were reversible when Diamox was withdrawn.